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Alpha-amylase supplementation improves broiler performance and intestinal health under reduced metabolizable energy conditions

A suplementação com alfa-amilase melhora o desempenho de frangos de corte e a saúde intestinal em condições de energia metabolizável reduzida

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ABSTRACT

Alpha-amylase improves carbohydrate digestion, increasing the available energy to maximize the performance of broiler chickens. This study evaluated the inclusion of alpha-amylase in diets with metabolizable energy (ME) valorization on growth performance, carcass yield, and intestinal health of broiler chickens. A total of 640 male broiler chickens (Cobb 500) were distributed in a completely randomized design with four treatments and eight repetitions of 20 birds each. The treatments were: PC (positive control), NC (negative control with a reduction of 100 kcal ME per kg diet), 100g-AA (NC + 100 g alpha-amylase per kg of diet), and 200g-AA (NC + 200 g alpha-amylase per kg diet). At 42 days, broiler chickens in the PC and supplemented groups (100g-AA and 200g-AA) showed better ME utilization, and at 21 and 42 days, greater weight gain (P<0.05). Broiler chickens supplemented with 100g-AA and 200g-AA had better digestibility of dry matter, ash, and gross energy (P<0.05) at 42 days. The ileal digestibility of starch was higher in the 100g-AA and 200g-AA groups compared to the PC group (P<0.10) at 42 days. The concentration of butyric acid in the cecal content was higher in the 200g-AA group compared to the PC group (P<0.05) at 35 days. Supplementation of alpha-amylase in diets with reduced ME is a viable strategy to optimize the performance of broiler chickens.

Index terms: Energy; enzyme; fatty acids; growth; poultry.

RESUMO

A alfa-amilase melhora a digestão de carboidratos, aumentando a energia disponível para maximizar o desempenho dos frangos de corte. Este estudo avaliou a inclusão de alfa-amilase em dietas com valorização da energia metabolizável (EM) no desempenho de crescimento, rendimento de carcaça e saúde intestinal de frangos de corte. Um total de 640 frangos de corte machos de um dia de idade (Cobb 500) foram distribuídos em um delineamento inteiramente casualizado, com quatro tratamentos e oito repetições de 20 aves cada. Os tratamentos foram: PC (controle positivo), NC (controle negativo com redução de 100 kcal de EM por kg de dieta), 100g-AA (NC + 100 g de alfa-amilase por kg de dieta) e 200g-AA (NC + 200 g de alfa-amilase por kg de dieta). Aos 42 dias, os frangos de corte nos grupos PC e suplementados (100g-AA e 200g-AA) apresentaram melhor utilização da EM, e aos 21 e 42 dias, maior ganho de peso (P<0,05). Os frangos suplementados com 100g-AA e 200g-AA tiveram melhor digestibilidade de matéria seca, cinzas e energia bruta (P<0,05) aos 42 dias. A digestibilidade ileal do amido foi maior nos grupos 100g-AA e 200g-AA em comparação ao grupo PC (P<0,10) aos 42 dias. A concentração de ácido butírico no conteúdo cecal foi maior no grupo com 200g-AA em comparação ao grupo PC (P<0,05) aos 35 dias. A suplementação de alfa-amilase em dietas com EM reduzida é uma estratégia viável para otimizar o desempenho dos frangos de corte.

Termos de indexação: Energia; enzima; ácidos graxos; crescimento; aves.

Introduction

Starch, the primary energy source in broiler diets, is crucial for the energy availability of the birds (Svihus et al., 2014; Sun et al., 2019). However, its digestion can be incomplete in the small intestine, varying based on feed ingredients and corn quality (Clarke et al., 2018; Petry et al., 2020; Duarte et al., 2021; Perz et al., 2023).

To enhance starch digestibility and optimize energy utilization, the inclusion of alpha-amylase has emerged as an effective strategy. This enzyme catalyzes the hydrolysis of complex starch molecules into smaller units, such as glucose, maltose, and dextrins, thereby facilitating their absorption in the gastrointestinal tract (Torres-Pitarch et al., 2019; Herwig et al., 2020; Genova et al., 2021). Previous studies have demonstrated that incorporating alpha-amylase into corn and soybean meal-based diets not only improves starch digestibility but also significantly enhances energy digestibility in broiler chickens, thereby contributing to more efficient energy utilization

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(Cowieson, Vieira, & Stefanello, 2019; Stefanello et al., 2019; Woyengo et al., 2019). However, the effectiveness of alpha-amylase supplementation is not uniform and is subject to a range of variable factors (Stefanello et al., 2019). The quality of feed ingredients, bird age, and individual characteristics are elements that directly influence the outcomes (Stefanello et al., 2015; Yuan et al., 2017; Massuquetto et al., 2020).

In this context, the evaluation of the incorporation of alpha-amylase into diets for broilers should not be limited to a mere analysis of its efficacy but should also encompass an understanding of how birds utilize nutrients (Woyengo et al., 2019; Perz et al., 2023). This process significantly impacts the performance of birds, including weight gain and feed efficiency (Cowieson et al., 2020; Yi et al., 2021). At the same time, it should be considered that, currently, poultry nutrition has reduced the addition of crude protein in diets supplemented with synthetic amino acids, resulting in an increased amount of corn in the formulations and, consequently, in the starch content (Alagawany, Elnesr, & Farag, 2018). This increase in starch content may exceed the digestive capacity of the gastrointestinal tract (Balasubramanian et al., 2020; Stefanello et al., 2023).

Furthermore, the assessment of intestinal health is also important, as it indicates how the addition of alpha-amylase in diets can impact nutrient absorption (Herwig et al., 2020; Stefanello et al., 2023). The integrity of the intestinal mucosa and the balance of the microbiota are crucial for effective digestion and absorption, directly influencing the growth and well-being of the poultry (Jiang et al., 2008; Kaczmarek et al., 2014; Woyengo et al., 2019; Perz et al., 2023). Thus, the presence of volatile fatty acids in the intestine acts as an important indicator of intestinal health, and their levels can be used to evaluate this health status, playing a vital role in maintaining the balance within the gut (Woyengo et al., 2019; Sun et al., 2020; Herwig et al., 2020; Perz et al., 2023).

In this context, fatty acids help preserve the integrity of the intestinal barrier, preventing the translocation of pathogens and toxins into the bloodstream (Schramm et al., 2021; Ravindran et al., 2021). These compounds also influence the composition of the gut microbiota, promoting beneficial bacteria while inhibiting the growth of pathogens (Woyengo et al., 2019; Sun et al., 2020). This microbial balance is essential for efficient digestion and nutrient absorption, further supporting overall intestinal health (Jiang et al., 2008; Kaczmarek et al., 2014; Perz et al., 2023). Furthermore, short-chain fatty acids possess anti-inflammatory properties that modulate both local and systemic immune responses by reducing pro-inflammatory cytokine production and enhancing the activity of regulatory immune cells (Herwig et al., 2020; Perz et al., 2023).

Building on the core hypothesis that incorporating alpha-amylase into diets to enhance starch digestion may facilitate a reduction in metabolizable energy formulation within the feed. In this way, the study's objective was to acess growth, metabolism and intestinal health of broiler chickens supplemented with alpha-amylase.

Material and Methods

Ethics Committee

The procedures described here were approved by the Ethics Committee for the Use of Animals of the National Council for Animal Control and Experimentation – UNIOESTE (Protocol nº 05/2022) and were previously approved by the National Council for Animal Control and Experimentation according to Normative No. 37 of February 15, 2018.

Enzyme

The alpha-amylase enzyme (Sunamy Amylase®, Wuhan City, Hubei, China) was produced through the fermentation of *Bacillus subtilis*, with a minimum activity of 1000 U/g and in vitro solubility of 48.5% and 34.4% in acidic and alkaline solutions, respectively.

Broiler chickens and diets

A total of 640 male Cobb 500 broiler chickens, all one day old, with an average weight of 47.09 ± 0.78 g, were distributed within a fully randomized experimental design comprising 4 treatment groups, each replicated 8 times, and containing 20 birds in each replication. The treatments were composed of: PC: positive control feed; NC: negative control with a reduction of 100 kcal metabolizable energy per kg of diet; 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet; 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. The positive control (PC) and negative control (NC) diets were formulated to meet the nutritional requirements of the birds, except for the metabolizable energy values in the NC diets. The inclusion of alpha-amylase in the diets replaced the inert material (kaolin).

The diets were formulated based on corn and soybean meals, according to the nutritional requirements established by Rostagno et al. (2024). The poultry received feed and water *ad libitum* throughout the experimental period. Percentages and composition of experimental diets expressed on a natural matter (Table 1). Digestible values determined from finishing rations expressed on a dry matter basis (Table 2).

Experimental period

The poultry used in the study were housed in a masonry aviary with a concrete floor, which was equipped with ventilation under negative pressure in the form of a tunnel and cooling through an evaporative plate. The aviary used for the experiment measured 25 meters in length and 8 meters in width and was divided into boxes of 1.76 m², with each box containing a tubular feeder, nipple drinker and 250 watts of electrical resistance as a heating source. Before the birds' arrival, the floor was covered with pine shavings which had been reused by five previous batches. The lighting program followed the recommendations of the manual (COBB 500, 2019), and the average, minimum and maximum temperature and relative humidity were monitored daily.

Table 1: Percentages and composition of experimental diets expressed on a natural matter basis.

Item (g/kg)	1 to 21 days		22 to 35 days		36 to 42 days	
	PC[1]	NC[2]	PC	NC	PC	NC
Corn 7.88%	554.02	564.72	565.96	589.32	570.70	593.97
Soybean meal 46%	343.26	341.40	295.01	290.91	239.73	235.63
Wheat bran	50.00	50.00	70.00	70.00	100.00	100.00
Soybean oil	15.95	1.00	38.11	18.86	52.31	33.07
Limestone (36%)	11.33	11.34	10.45	10.48	9.65	9.67
Dicalcium phosphate (18%)	10.62	10.61	8.15	8.13	5.58	5.55
Sodium chloride	4.38	4.27	3.83	3.82	3.37	3.36
Lysine sulfate (60%)	3.71	3.76	2.92	3.04	3.46	3.58
DL- methionine (99%)	3.11	3.10	2.41	2.28	2.11	2.08
L-threonine (99%)	1.07	1.07	0.71	0.71	0.74	0.74
Adsorbent[3]	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50
Inert[6]	0.20	6.38	0.20	0.20	0.20	0.20
Choline chloride 60%	0.50	0.50	0.40	0.40	0.30	0.30
Phytase	0.10	0.10	0.10	0.10	0.10	0.10
Celite					10.00	10.00
Nutritional composition						
Met. energy (kcal kg^{-1})	2950	2850	3100	3000	3200	3100
Crude protein (g kg^{-1})	220.0	220.0	200.0	200.0	180.0	180.0
Starch (g kg^{-1})	416.46	423.87	429.57	445.84	447.60	463.87
Crude fiber (g kg^{-1})	37.76	37.85	36.69	36.90	36.13	36.33
Available phosphorus (g kg^{-1})	4.50	4.50	4.00	4.00	3.50	3.50
Dig. lysine (g kg^{-1})	12.00	12.00	10.50	10.50	9.50	9.50
Dig. meth. + cyst. (g kg^{-1})	8.88	8.88	7.77	7.77	7.03	7.03
Dig. threonine (g kg^{-1})	7.92	7.92	6.93	6.93	6.27	6.27
Dig. tryptophan (g kg^{-1})	2.38	2.38	2.15	2.14	1.90	1.90
Calcium (g kg^{-1})	9.00	9.00	8.00	8.00	7.00	7.00
Total choline (mg kg^{-1})	16.16	16.171	14.61	14.63	12.99	13.02
Sodium (g kg^{-1})	2.00	2.00	1.80	1.80	1.60	1.60
Chlorine (g kg^{-1})	3.16	3.17	2.87	2.88	2.58	2.59

[1]PC: Positive control feed.
[2]NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet.
[3]Bentonite.
[4]Vitamin supplement, composition per kg of product: Vit. A (min) 32000 IU; Vit. D_3 (min) 8000 IU; Vit. E (min) 60000 IU; Vit. K3 (min) 6000 mg; Vit. B1 (min) 7200 mg; Vit B2 (min) 20000 mg; Vit. B6 (min) 9600 mg; Vit. B12 (min) 40000 mcg; pantothenic acid (min) 36000 mg; Niacin (min) 108000 mg; folic acid (min) 3300 mg; biotin (min) 160 mg; BHT 290 mg.
[5]Mineral supplement, composition per kg of product: Copper (min) 16 g; Iron (min) 100 g; Manganese (min) 140 g; Zinc (min) 120 g; Iodine (min) 1600 mg; Selenium (min) 600 mg.
[6]Inert: The inert used was kaolin-based, with the inclusion of the α-amylase (kg ton^{-1}).

Table 2: Digestible values determined from finishing rations expressed on a dry matter basis.

[1]Treatments	[2]DM	[3]CP	[4]EE	Starch	Ash	[5]CE
	(%)	(%)	(%)	(%)	(%)	(kcal/kg)
PC	89.9	19.9	8.1	48.9	6.0	4771
NC	89.9	19.6	6.3	51.0	5.6	4578
100g-AA	89.4	19.3	6.3	51.0	6.0	4598
200g-AA	89.9	19.4	6.3	51.2	6.2	4571

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. [2]DM: Dry Matter. [3]CP: Crude protein. [4]EE: Ether extract. [5]CE: Crude energy.

Performance and carcass traits

Feed intake measurements were conducted at 21 and 42 days of age to evaluate weight gain (WG), feed intake (FI), and feed conversion ratio (FCR). Daily mortality was also monitored, and feeders were weighed to ensure accurate calculations of feed intake and feed conversion ratio, following the methodology described by Sakomura and Rostagno (2016).

At 21 and 42 days of age, the efficiency of energy utilization (EEU) was calculated using the formula EEU = (average feed intake x dietary energy) /1000. Subsequently, the efficiency of metabolizable energy utilization (EMEU) was determined by dividing EEU by the average weight gain (AWG), according to the formula: EMEU= EEU /AWG.

At 42 days of age, the productive efficiency index (PEI) was calculated using the following formula: PEI = (Average weight in kg) x (100 - Mortality percentage, %) / (Slaughter age in days) x Feed conversion) x 100. Livability percentage was calculated using the following formula: Livability % = (Number of birds sold x 100) / Number of birds at the beginning.

At 42 days of age, two poultry per experimental unit were randomly selected, weighed, marked, and slaughtered due to electronarcosis. The carcasses were weighed and stripped, obtaining legs (thigh and drumstick), wings, breast fillet, which were weighed individually. The liver, pancreas, and abdominal fat (consisting of the adipose tissue around the cloaca, gizzard, proventriculus and adjacent abdominal muscles) were separated and weighed to determine their weight relative to the live weight of the poultry. Carcass yield was determined by the clean and eviscerated carcass weight in relation to the live weight of the poultry. The cut yield was determined by the ratio between the weight of the cuts and the weight of the cleaned and eviscerated carcass.

Nutrient digestibility

At 35 days of age, all birds received a silica source (Celite®) added to their experimental diets at a proportion of 1% as an indigestible marker. The birds underwent a seven-day adaptation period with the marker. After the adaptation period to the diets, two birds per experimental unit, with an average weight (± 5%), were individually weighed and euthanized by cervical dislocation followed by bleeding. One of the birds was used for organ collection.

To evaluate the digestibility of nutrients, present in the ileal content, the material was collected from the intestinal tract of birds. The removal of the intestinal content, the ileal fraction, was established 4 cm below the Meckel's diverticulum and 4 cm above the ileocecal-colonic junction. The digesta samples were homogenized and immediately sent for drying in a circulating air oven at 55°C for 72 hours. After drying, the samples were ground, and together with the samples from the experimental diets, they were sent for analysis of dry matter (DM), gross energy (GE), crude protein (CP), and acid insoluble ash (AIA).

Protein was analyzed using the Kjeldahl method, and dry matter (definitive drying) was determined according to AOAC methodology (2000), methods 984.13 and 935.29, respectively. For the determination of gross energy, the samples were pelleted and subjected to combustion in a calorimetric bomb (Model C2000. Control, Ike Werke). The starch analyses were conducted using the AOAC International method 996.11 (AOAC, 2000). The fat content of the experimental diets and corn was analyzed according to the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 2000).

For the determination of insoluble acid ash (IAA), the ash content was first determined according to AOAC (2000), method 932.03. After combustion, the crucibles were placed in a metal form filled with sand for better heat propagation. In the fume hood, 10 mL of hydrochloric acid (4 mol L^{-1}) was added to each crucible, and the metal form was placed on a hot plate at 260°C until complete acid evaporation. The samples were left to cool, and then the crucibles were washed three times with deionized water, and the contents were filtered through quantitative filter paper. The filter paper from each sample was placed in new pre-weighed crucibles, and after drying in a circulating air oven (80°C), the burning process was repeated to obtain the ash content. The insoluble acid ash (IAA) value was obtained by difference in weight.

Using the laboratory results, the coefficients of digestibility (CD) for dry matter and crude protein were determined, as well as the values of digestible energy (DE) (Sakomura; Rostagno, 2016).

Intestinal morphometry

To evaluate intestinal morphometry parameters such as villus height, crypt depth, and villus height: crypt depth ratio, one bird from each experimental group was euthanized by cervical dislocation at 35 days of age. The digestive tract was collected for analysis, and a 5 cm segment of the jejunum located before Meckel's diverticulum was selected. The duodenum segment considered for sampling was the distal portion of the duodenal loop to Meckel's diverticulum after exposing the small intestine.

The tissue fragments were fixed in a 10% buffered formalin solution, dehydrated in a graded series of ethanol, and then embedded in paraffin. Semi-serial sections of 5 µm were prepared from each segment and mounted on glass slides. The sections were then stained with the hematoxylin-eosin technique following the protocol described by Luna (1968).

Short-chain fatty acids in cecal contents

The quantification of short-chain fatty acids (SCFA) was carried out on cecal content collected from one bird per pen at 35 days of age, following the method described by Del Valle et al. (2018). The cecal content was removed and 200 mg were weighed and transferred to 2 mL microtubes (CRAL, São Paulo, Brazil) properly identified.

A total of 1800 µL of 1% (w/v) NaOH solution was added to the cecal content. Then, the microtubes were homogenized in a multifunctional vortex (Kasvi Brand, K40-1010, Paraná, Brazil) for 2 min at 3000 g. After homogenization, the microtubes were centrifuged (Kasvi Brand, K14-4000, Paraná, Brazil) at 1050 g for 5 min, to completely sediment the solid fraction of the sample.

A volume of 900 µL of the supernatant was transferred (Single-channel Micropipette Plus 100 - 1000 µL, Kasvi, K1 - P1000, Paraná, Brazil) to new microtubes (CRAL, São Paulo, Brazil) and acidified with 50 µL (Single-channel Micropipette Plus 10 - 100 µL, Kasvi, K1 - P100, Paraná, Brazil) of 50% (w/v) ortho-phosphoric acid solution. The acidified samples were homogenized (Kasvi Brand, K40-1010, Paraná, Brazil) for 30 sec at 3000 g and stored in a freezer at -20 °C until analysis.

The concentrations of acetic, propionic, butyric, valeric, and isovaleric acids in the samples were determined by gas chromatography using a Shimadzu© GC-2010 Plus chromatograph equipped with an AOC-20i automatic injector, a Stabilwax-DA™ capillary column (30m, 0.25 mm ID, 0.25 µm df, Restek©), and a flame ionization detector (FID) after acidification with 1 M p.a. o-phosphoric acid (Ref. 100573, Merck©) and fortification with a mixture of free volatile acids (Ref. 46975, Supelco©).

A 1 µL aliquot of each sample was injected with a split ratio of 40:1, using helium gas as the carrier with a linear Velocity of 42 centimeters per second. The separation of analytes was achieved in a 12 min chromatographic run. The injector and detector temperatures were set at 250 ºC and 300 ºC, respectively, and the initial column temperature was 40 ºC. The column temperature gradient began with an increase from 40 ºC to 120 ºC at a rate of 40 ºC per minute, followed by an increase from 120 ºC to 180 ºC at a rate of 10 ºC per minute, and from 180 ºC to 240 ºC at a rate of 120 ºC per minute. The temperature was maintained at 240ºC for an additional 3 minutes at the end.

For analyte quantification, method calibration was performed with dilutions of WSFA-2 standard (Ref. 47056, Supelco®) and glacial acetic acid (Ref. 33209, Sigma-Aldrich®) analyzed under the conditions described above. Peak determination and integration were performed using the GCsolution software v. 2.42.00 (Shimadzu©, Paraná, Brazil). The results were expressed in mmol kg^{-1}.

Statistical analyses

The data were checked for normality of residuals, presence of outliers (PROC UNIVARIATE and Shapiro-Wilk) and variance analysis. If significant effects were observed, the Tukey test was conducted at both 5% and, if deemed necessary, 10% significance levels to compare the means. All statistical procedures were performed using the SAS statistical program (SAS, 2014).

Results and Discussion

At 21 days of age (Table 3), broiler chickens supplemented with 100g-AA and 200g-AA of alpha-amylase showed feed intake, weight gain, and feed conversion rates similar to those of the NC group and inferior to those of the PC group ($P<0.05$).

However, at 42 days of age (Table 4), broiler chickens supplemented with 100g and 200g of alpha-amylase exhibited weight gain and feed conversion ratios similar to those of the PC group ($P<0.05$). Also, broiler chickens supplemented with alpha-amylase (100g-AA and 200g-AA) exhibited productivity efficiency indices (482 and 459, respectively) higher than those of the NC group (430), but lower than those of the PC group (506) ($P<0.05$).

The results of metabolizable energy utilization efficiency indicated that birds receiving alpha-amylase (100g-AA and 200g-AA), and PC showed better utilization of metabolizable energy (3.82, 3.83, and 3.85, respectively) at 21 days of age ($P<0.05$). On the other hand, birds in the NC group consumed a higher quantity of metabolizable energy that did not convert into weight gain (3.95). At 42 days of age, a similar pattern was observed in Metabolizable Energy Utilization Efficiency, indicating that birds receiving alpha-amylase (100g-AA and 200g-AA) and PC demonstrated better utilization of metabolizable energy (4.74, 4.78, and 4.81, respectively), resulting in higher weight gain ($P<0.05$). Conversely, birds in the NC group consumed a higher quantity of metabolizable energy that did not convert into weight gain (5.05).

Table 3: Performance of 21-day-old broiler chickens fed diets supplemented with or without alpha-amylase.

[1]Treatments	Feed intake (g)	Weight gain (g)	Feed conversion ratio (g g^{-1})	[2]EMEU
PC	1301[a]	997[a]	1.306[a]	3.85[a]
NC	1263[b]	923[b]	1.369[b]	3.95[b]
100g-AA	1261[b]	940[b]	1.340[b]	3.82[a]
200g-AA	1245[b]	930[b]	1.340[b]	3.83[a]
[3]SEM	31.24	19.92	0.02	1.63
[4]P value	0.001	0.001	0.001	0.004

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. [2]EMEU: Efficiency of metabolizable energy utilization. [3]SEM: The standard error of the mean. [4]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%.

Table 4: Performance of 42-day-old broiler chickens fed diets supplemented with or without alpha-amylase.

[1]Treatments	Feed intake (g)	Weight gain (g)	Feed conversion ratio (g g^{-1})	[2]EMEU	[3]PEI	Livability (%)
PC	5282	3366[a]	1.565[a]	4.81[a]	506[a]	97.50
NC	5198	3131[b]	1.647[b]	5.05[b]	430[c]	96.25
100g-AA	5187	3280[a]	1.595[a]	4.74[a]	482[ab]	97.50
200g-AA	5235	3282[a]	1.595[a]	4.78[a]	459[b]	93.75
[4]SEM	77.17	63.17	0.03	2.07	20.14	3.13
[5]P value	0.08	0.001	0.001	0.001	0.001	0.080

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g of alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g of alpha-amylase kg^{-1} diet. [2]EMEU: Efficiency of metabolizable energy utilization. [3]PEI: Production efficiency index. [4]SEM: The standard error of the mean. [5]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%.

The performance results of the birds at 21 days of age suggested that the positive control provided the best condition in terms of weight gain compared to the other treatments. However, the diets supplemented with alpha-amylase achieved a feed conversion ratio similar to the positive control group, indicating that this enzyme had a positive effect on nutrient utilization, especially starch, even under conditions of energy restriction, as reported by Yin et al. (2018), Zhou et al. (2021), Bassi et al. (2023).

In the intestine, alpha-amylase acts in a complementary manner to glucoamylase, which further breaks down oligosaccharides and dextrins into free glucose molecules, essential for the efficient absorption of energy by the organism (Cihan et al., 2018; Aderibigbe et al., 2020). This combined process ensures the complete degradation of starch, maximizing carbohydrate digestion. Thus, the supplementation with amylase and the subsequent action of glucoamylase contribute to better utilization of dietary starch (Oliveira et al., 2018; Zhou et al., 2021).

The supplementation of alpha-amylase (100g-AA and 200g-AA) provided the birds with an enhanced capacity to harness the energy present in their diet. Simular results were observed by Teixeira et al. (2019) and Aderibigbe et al. (2020). This is due to alpha-amylase's ability to break down the carbohydrates present in the birds' diet into smaller units, facilitating the absorption and efficient utilization of energy by their bodies (Alagawany, Elnesr, & Farag, 2018; Castro et al., 2019). With this more effective breakdown of nutrients, the birds were able to extract a greater amount of energy from the consumed food, allowing them to redirect this extra energy towards body growth and development (Stefanello et al., 2019; Bassi et al., 2023). As a result, at 42 days of age, they achieved a weight gain comparable to that of the group of birds that received PC.

The improvement in weight gain and feed conversion ratio in birds that received alpha-amylase (100g-AA and 200g-AA) in diets enriched with energy, at 42 days of age, is consistent with studies such as those by Yin et al. (2018), Zhou et al. (2021), Massuquetto et al. (2020), Bassi et al. (2023), that also observed benefits in growth performance when supplementing poultry diets with alpha-amylase In fact, the results of the present study are likely associated with the ability of the enzyme alpha-amylase to hydrolyze starch, a polysaccharide primarily composed of amylose and amylopectin, into oligosaccharides and disaccharides, such as maltose and maltotriose, which are more easily digested, absorbed, and utilized by birds (Onderci et al., 2006; Clarke et al., 2018; Pirgozliev, Rose, & Ivanova,

2019). In addition to starch, alpha-amylase can also hydrolyze other polysaccharides containing α-1,4 glycosidic bonds, such as glycogen (Zhu et al., 2014; Kim et al., 2017).

However, it is unable to break the α-1,6 bonds that occur in the branches of amylopectin and glycogen, requiring the action of other enzymes, such as isoamylase and debranching enzyme, to complete digestion (Alagawany, Elnesr, & Farag, 2018; Zho et al., 2021). These additional enzymes are essential for the effective degradation of branched polysaccharides, allowing the products of digestion to be rapidly available for absorption in the gastrointestinal tract Woyengo et al. (2019). Therefore, as indicated by Stefanello et al. (2015), Yuan et al. (2017), Woyengo et al. (2019), and Bassi et al. (2023), the action of alpha-amylase may have facilitated the digestion of complex carbohydrates present in the diet, increasing the availability of essential nutrients for the birds. Thus, it is likely that this synergistic interaction between alpha-amylase and other digestive enzymes contributed to the improvements in weight gain and feed efficiency observed in our study, corroborating previous findings.

Carcass yield was higher (P<0.05) in birds that received the PC diet, while birds that received diets with alpha-amylase and NC had the lowest carcass yield (Table 5).

The reduction in carcass yield observed in the groups of birds that received the negative control and alpha-amylase supplementation (100g-AA and 200g-AA) can be explained by specific physiological and metabolic factors related to energy restriction in the diet, as reported by Gracia et al. (2003), Alagawany, Elnesr and Farag, (2018), and Zho et al. (2021). Energy restriction leads to a decrease in the availability of essential nutrients, which can affect muscle development in several ways (Alagawany, Elnesr, & Farag, 2018; Zho et al., 2021).

Firstly, limited energy availability can impair protein synthesis, which is essential for muscle growth and repair, resulting in lower deposition of muscle tissue (Yuan et al., 2017; Woyengo et al., 2019). Secondly, under energy-restricted conditions, birds may prioritize energy for vital functions overgrowth, leading to a reduction in the resources allocated for muscle development (Zho et al., 2021). Moreover, energy restriction can stimulate catabolic processes, where the body breaks down muscle tissue for energy, further contributing to the reduction in carcass yield (Bedford & Cowieson, 2012; Alagawany, Elnesr, & Farag, 2018). These factors collectively result in a decrease in carcass yield, as evidenced by the lower deposition of muscle tissue observed in this study (Massuquetto et al., 2020).

The coefficient of digestibility for ash was similar in the groups that received diets with alpha-amylase and PC, while NC showed the lowest result (P<0,05). The coefficient of digestibility for gross energy was higher (P<0.05) in birds that received diets with alpha-amylase, while PC and NC showed the lowest result (Table 6).

The digestible starch was higher (P<0.10) in birds that received diets supplemented with alpha-amylase compared to those that received PC and NC diets. The digestible ash and digestible energy were higher (P<0.05) in birds that received PC and diets with alpha-amylase. Digestible fat was higher (P<0.05) in birds that received PC and lower in the groups that received NC and diets with alpha-amylase (Table 7).

The digestibility coefficients of starch and digestible starch revealed that birds exhibited similar efficiency in digesting this component. However, it is relevant to emphasize that both treatments, including the negative control and groups subjected to alpha-amylase supplementation, experienced a significant reduction of 100 kcal in metabolizable energy.

This scenario suggests that, to compensate for this energy decrease, birds may have implemented mechanisms for mobilizing additional resources or metabolic adjustments to meet the energy demands imposed by caloric restriction (Stefanello et al., 2019). The present study highlighted a physiological adaptation to energy restriction, suggesting that alpha-amylase supplementation played a potentially crucial role in optimizing the digestion and efficient utilization of the available energy substrate in diets, as reported by Ravindran and Abdollahi, (2021), Stefanello et al. (2023).

Table 5: Carcass yield, cuts yield, and relative organ weight of broiler chickens at 42 days fed diets supplemented or not with alpha-amylase.

[1]Treatments	[2]CY	[3]WY	[4]BFY	[5]LY	[6]RWAF	[7]RLW	[8]RPW
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
PC	71.07[a]	9.51	28.53	33.10	1.45	1.64	0.18
NC	69.48[b]	9.54	28.48	32.88	1.46	1.67	0.17
100g-AA	69.77[b]	9.71	28.46	32.75	1.25	1.68	0.18
200g-AA	68.94[b]	9.75	28.50	32.26	1.57	1.79	0.17
[9]SEM	1.68	0.87	1.80	1.37	0.36	0.16	0.02
[10]P value	0.001	0.833	0.991	0.371	0.111	0.112	0.393

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g of alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g of alpha-amylase kg^{-1} diet. [2]CY: Carcass yield. [3]Wy: Wing yield. [4]BFY: Breast fillet yield. [5]LY: Leg yield (drums and thighs). [6]RWAF: Relative weight of abdominal fat. [7]RLW: Relative liver weight. [8]RPW: Relative pancreas weight. [9]SEM: The standard error of the mean. [10]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%.

Table 6: Ileal digestibility coefficient of broiler chicken at 42 days fed diets supplemented or not with alpha-amylase.

[1]Treatments	[2]CPDC (%)	[3]EEDC (%)	[4]SDC (%)	[5]DMDC (%)	[6]ADC (%)	[7]GEDC (%)
PC	79.19	83.71	94.31	75.03[b]	92.91[a]	76.6[b]
NC	80.35	84.44	93.04	75.90[b]	92.31[b]	76.9[b]
100g-AA	80.41	85.37	94.18	77.29[a]	93.16[a]	79.1[a]
200g-AA	79.91	83.45	93.96	77.23[a]	93.42[a]	78.8[a]
[8]SEM	1.93	2.44	0.51	0.97	0.49	1.10
[9]P value	0.802	0.702	0.843	0.021	0.042	0.001

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. [2]CPDC: Crude protein digestibility coefficient. [3]EEDC: Ether extract digestibility coefficient. [4]SDC: Starch Digestibility Coefficient. [5]DMDC: Dry matter digestibility coefficient. [6]ADC: Ash digestibility coefficient. [7]GEDC: Gross energy digestibility coefficient. [8]SEM: The standard error of the mean. [9]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%.

Table 7: Ileal digestibility of nutrients expressed on a dry matter basis in broiler chickens at 42 days of age.

[1]Treatments	[2]DP (%)	[3]DEE (%)	[4]DS (%)	[5]DDM (%)	[6]DA (%)	[7]DE (kcal/kg)
PC	15.8	6.7[a]	46.1[B]	67.7	6.2[b]	3653[a]
NC	15.5	5.3[b]	47.4[B]	68.9	5.7[c]	3521[b]
100g-AA	15.5	5.4[b]	48.0[A]	70.2	6.3[a]	3637[a]
200g-AA	15.5	5.3[b]	48.1[A]	69.6	6.5[a]	3620[a]
[8]SEM	0.32	0.16	0.32	1.21	0.03	50.83
[9]P value	0.582	0.001	0.082	0.102	0.001	0.001

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. [2]DP: Digestible protein. [3]DEE: Digestible ether extract. [4]DS: Digestible starch. [5]DMM: Digestible dry matter. [6]DA: Digestible ash. [7]DE: Digestible energy. [8]SEM: The standard error of the mean. [9]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%. [A,B,C]: Means followed by different letters are statistically different according to Tukey test at 10%.

The improvement in ash digestibility was crucial for the weight gain improvement in birds fed alpha-amylase (100g-AA and 200g-AA), as ash play vital roles in various physiological processes, including tissue formation and maintenance, and immune function (Selle, Liu, & Cowieson, 2012; Stefanello et al., 2019). Moreover, the enhanced digestibility of ash indicated that the birds were able to absorb and utilize these essential nutrients more efficiently, contributing to better growth and development (Pirgozliev, Rose, & Ivanova, 2019; Schramm et al., 2021; Ravindran & Abdollahi, 2021).

The improvement in dry matter digestibility is a promising result because it indicates greater efficiency in the absorption of essential nutrients, such as carbohydrates, proteins, and lipids, present in the diets of broiler chickens (Aderibigbe et al., 2020; Stefanello et al., 2023). This suggests that the inclusion of alpha-amylase in the diet facilitated the breakdown of complex carbohydrates, making them more accessible for digestion (Stefanello et al., 2019; Schramm et al., 2021). This increased digestibility not only enhances the utilization of available nutrients but may also lead to improved metabolic performance, resulting in increased weight gain and feed conversion, as observed in the study (Aderibigbe et al., 2020). Also, the improvement in the coefficient of digestibility of crude energy and digestible energy suggested that alpha-amylase supplementation (100g-AAand 200g-AA) ensured better utilization of available energy in the diets. Similar results were found by Kaczmarek et al. (2014), Stefanello et al. (2019), Ravindran and Abdollahi, (2021) and Zhou et al. (2021).

When evaluating the digestibility of starch concerning weight gain at 42 days, it becomes apparent that the enhanced starch digestibility sufficed to ensure comparable performance among treatments enriched with energy and supplemented with amylase. However, this effect isn't evident at 21 days due to heightened protein anabolism in young birds, leading to increased energy expenditure. Consequently, the results underscore that incorporating alpha-amylase into broiler diets can serve as an effective strategy to enhance the digestibility of essential nutrients (Yuan et al., 2017; Stefanello et al., 2023). This enhancement in digestibility may translate into improved nutrient absorption and utilization, thereby fostering advantages such as enhanced weight gain and productivity efficiency (Alagawany, Elnesr, & Farag, 2018; Schramm et al., 2021).

In the duodenum, birds that received the 200g-AA diet had a greater crypt depth (P<0.05) than the group that received PC, while birds that received NC and 100g-AA had the smallest crypt depth. Villus height was higher (P<0.05) in birds that received PC, NC, and 200g-AA supplementation. The villus-to-crypt ratio was higher (P<0.05) in birds that received NC, while in the groups that received diets supplemented with alpha-amylase, the villus-to-crypt ratio was lower and similar to PC. In the jejunum, there were no significant differences (P>0.05) in crypt depth, villus height, and villus-to-crypt ratio among the treatments (Table 8).

The depth of crypts, villus height, and the villus:crypt ratio observed in this study are consistent with other studies that have investigated the effects of alpha-amylase supplementation on the histological characteristics of the intestinal tract in animals (Bedford et al., 2012; Ravindran et al., 2016; Amerah et al., 2017). A study conducted by Oliveira et al. (2018) evaluated the effects of alpha-amylase supplementation in diets for broiler chickens and found significant differences in crypt depth and villus height in the duodenum. The animals supplemented with alpha-amylase exhibited shallower crypts and shorter villi height compared to the control group, indicating lower cell turnover in the intestinal villi, which according to Stefanello et al. (2023), characterizes better intestinal villi epithelium health

Another study conducted by Teixeira et al. (2019) investigated the effects of alpha-amylase supplementation in diets for pigs and observed significant differences in crypt depth and villus height in the duodenum. The pigs supplemented with alpha-amylase exhibited shallower crypts and lower villus height compared to the control group.

The results of the present study indicated that alpha-amylase supplementation can alter the histological characteristics of the intestinal tract, especially in the duodenum, corroborating with Zimonja and Svihus, (2009) and Yuan et al. (2017). The shallower crypt depth and lower villus height observed in the groups supplemented with alpha-amylase (100g-AA) may indicate an improvement in nutrient absorption and intestinal health. These results align with previous findings by Cowieson et al. (2019) and Schramm et al. (2021).

Aderibigbe et al. (2020) also observed modifications in intestinal morphology in response to alpha-amylase supplementation, characterized by an increase in villus length and crypt depth. The authors of this study suggested that these changes are associated with an improvement in nutrient absorption, reinforcing the evidence that alpha-amylase supplementation can induce positive alterations in the intestinal tract.

Onderci et al. (2006) add an additional dimension to this understanding. They not only attributed the increase in villus length and crypt depth to the presence of exogenous amylase but also emphasized the influence of the age progression of the birds. This multifactorial perspective emphasizes the complexity of the factors contributing to intestinal morphological changes, encompassing not only specific supplementation but also intrinsic factors such as the age of the birds. However, from these correlations between different studies, it can be inferred that alpha-amylase supplementation not only triggers adjustments in intestinal morphology but also suggests a positive impact on nutrient absorption capacity (Jiang et al., 2008; Kaczmarek et al., 2014; Yuan et al., 2017).

Concentrations of acetic and valeric acids were higher (P<0.05) in birds that received diets with alpha-amylase and PC, and lower in birds that received the NC diet. The concentration of butyric acid was higher (P<0.05) in birds that received the 200g-AA diet, while the NC group exhibited the lowest concentration of butyric acid (Table 9).

The supplementation of alpha-amylase (200g-AA) in the present study demonstrated a significant impact on the profile of volatile fatty acids when compared to birds fed the negative control. Acetic acid is recognized as an important energy source for intestinal cells and can promote intestinal health (Yi et al., 2021). On the other hand, butyric acid has been associated with anti-inflammatory effects and benefits for the gastrointestinal tract (Scanes & Pierzchala-Koziec, 2014).

Table 8: Intestinal morphometry of the jejunum and duodenum of broiler chickens at 35 days of age fed diets supplemented or not with alpha-amylase.

[1]Treatments	Duodenum			Jejunum		
	[2]CD (*μm*)	[3]VH (*μm*)	[4]VCR	CD (*μm*)	VH (*μm*)	VCR
PC	136.89[b]	1054.44[a]	7.71[b]	122.23	715.24	5.83
NC	108.55[c]	1036.95[a]	9.55[a]	125.52	723.62	5.76
100g-AA	110.72[c]	854.83[b]	7.74[b]	132.01	735.72	5.58
200g-AA	153.54[a]	1033.71[a]	6.75[b]	129.00	717.71	5.55
[5]SEM	8.00	40.91	0.35	8.59	97.17	0.59
[6]P value	0.001	0.001	0.001	0.232	0.905	0.602

[1]Treatments: PC: Positive control feed. NC: Negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. [2]CD: Crypt Depth. [3]VH: Villus Height. [4]VCR: Villus-to-Crypt Ratio. [5]SEM: The standard error of the mean. [6]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%.

Table 9: Concentration of short-chain fatty acids in the cecal content of broiler chickens at 35 days of age fed diets supplemented or not with alpha-amylase.

[1]Treatments	Propanoic	Isobutyric	Isovaleric	Acetic	Valeric	Butyric
	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}
PC	4.03	0.35	0.52	40.00[a]	0.50[a]	7.71[b]
NC	3.49	0.40	0.53	30.07[b]	0.37[b]	5.07[c]
100g-AA	4.20	0.33	0.50	34.55[ab]	0.50[a]	7.66[b]
200g-AA	4.06	0.23	0.39	38.60[a]	0.50[a]	9.25[a]
[2]SEM	0.08	0.02	0.03	0.82	0.02	0.35
[3]P value	0.263	0.213	0.493	0.001	0.010	0.001

[1]Treatments: PC: positive control feed; NC: negative control with a reduction of 100 kcal of metabolizable energy per kg of diet. 100g-AA: NC + 100 g alpha-amylase kg^{-1} diet. 200g-AA: NC + 200 g alpha-amylase kg^{-1} diet. [2]SEM: The standard error of the mean. [3]P value (0.05%). [a,b,c]: Means followed by different letters are statistically different according to Tukey test at 5%.

The increase in the concentration of these acids suggests alterations in microbial fermentation and nutrient metabolism in birds fed supplemented alpha-amylase (Bedford & Cowieson, 2012; Zhou et al., 2021). These changes may be related to the ability of the alpha-amylase enzyme to break down starch complexes into simple sugar molecules, making them more readily fermentable by intestinal bacteria (Stefanello et al., 2015; Yuan et al., 2017; Cowieson et al., 2020).

In the study conducted by Munyaka et al. (2016) when working with different diets based on corn or wheat, with or without xylanase supplementation, they found that the type of diet influences the concentration of fatty acids in the cecal content. Wheat-based diets increased the production of acetic and butyric acid, while corn-based diets resulted in higher concentrations of propionic, valeric, and isovaleric acid in the cecal content of broiler chickens. Furthermore, the authors found that regardless of the diet type, the inclusion of xylanase led to a higher concentration of acetic acid, improved animal performance, and increased nutrient utilization, suggesting the hydrolysis of soluble and insoluble non-starch polysaccharides (PNAs).

Therefore, the results of the present study indicate the potential of alpha-amylase supplementation as a strategy to optimize intestinal health and performance in broiler chickens, algo reported by Aderibigbe et al. (2020), Schramm et al. (2021) and Bassi et al. (2023). However, it is important to emphasize the need for further studies to evaluate other parameters related to growth performance to obtain a more comprehensive understanding of the effects of alpha-amylase supplementation (Weurding, Enting, & Verstegen, 2003; Yin et al., 2018; Craig et al., 2020).

Conclusions

The inclusion of 100g to 200g of alpha-amylase per kg^{-1} of feed in reduced metabolizable energy diets is recommended as an effective strategy to improve the performance of broiler chickens. Supplementation has been shown to optimize starch digestion, increase energy utilization efficiency, and promote better feed conversion and greater weight gain, making it a viable alternative in energy-restricted diets.

Author Contributions

Conceptual idea: Nunes, R. V.; Ribeiro, T. P.; Methodology design: Nunes, R. V.; Data collection: Bruch, C. A.; Data analysis and interpretation: Rohloff Junior, N.; Writing and editing: Bruch, C. A.; Andrade, T. S.; Vargas Junior, J. G.

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